Environmental Signaling: A Biological Context for Endocrine Disruption

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Endogenous and exogenous chemical signals have evolved as a means for organisms to respond to physical or biological stimuli in the environment. Sensitivity to these signals can make organisms vulnerable to inadvertent signals from xenobiotics. In this review we discuss how various chemicals can interact with steroidlike signaling pathways, especially estrogen. Numerous compounds have estrogenic activity, including steroids, phytoestrogens, and synthetic chemicals. We compare bioavailability, metabolism, interaction with receptors, and interaction with cell-signaling pathways among these three structurally diverse groups in order to understand how these chemicals influence physiological responses. Based on their mechanisms of action, chemical steroid mimics could plausibly be associated with recent adverse health trends in humans and animals. — *Environ Health Perspect* 106(Suppl 1):5–10 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/5-10cheek/abstract.html*

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Environmental Signaling at the Organism Level

Sensitivity to chemical signals has evolved as a means by which organisms respond to changes in their environment. Internal chemical signaling is the means of communication between cells within an organism, while external chemical signaling, or environmental signaling, occurs when exogenous chemicals interact with internal signaling pathways. Presumably, responses to these signals are adaptive, allowing the organism to survive and reproduce. However, maladaptive or detrimental responses may also be elicited by external chemical signals.

Plants have evolved several effective methods of environmental signaling, including secretion of compounds that initiate the symbiosis between legumes and the nitrogen-fixing bacteria, *Rhizobium* spp. The secretion of flavonoids from legumes such as alfalfa and peas regulates the expression of bacterial gene products required for nodulation—the formation of plant root nodules containing the bacteria (*1–4*). Some flavonoids activate the nodulation process whereas others antagonize it, not unlike the agonistic and antagonistic activity of steroid hormones interacting with hormone receptors (*5*).

Not only do phytochemicals interact with signaling pathways in bacteria, they can also mimic mammalian hormones. Clover disease, an infertility syndrome found in sheep grazing on subterranean clover (*6*), has been linked to phytochemicals that function as estrogens and interfere with reproduction. These phytoestrogens have been suggested as a defense strategy by plants to limit the fertility of herbivores (*7*). Sensitivity to phytochemical hormone mimics could possibly be adaptive in herbivorous and omnivorous vertebrates. This sensitivity may have evolved as a means of linking reproductive timing to an adequate food supply.

Phytoestrogens may have beneficial effects in humans. Women and men living in Pacific Rim nations, where traditional soy diets are high in plant estrogens, have a lower incidence of breast and prostate cancer and of atherosclerotic cardiovascular disease than people in Western nations (*8–10*). Women in Australia who eat diets rich in phytoestrogens also have a lower incidence of breast cancer (*11*). The isoflavones present in soy appear to be responsible for these effects, possibly due to modulation of estrogen-signaling pathways. Interestingly, when Asian groups immigrate to the United States and switch to a more Western diet, their rates of breast cancer increase, although rates remain lower than those of the general U.S. population (*9*).

Environmental signaling by plants serves as a model for inadvertent signaling by synthetic chemicals. Recently, a number of synthetic chemicals (e.g. pesticides, detergents, and plasticizers) have been identified that can potentially modulate endocrine processes. The best studied examples are environmental estrogens—chemicals that mimic the activity of the natural sex steroid hormone, estradiol.

Estradiol-17β regulates reproduction in many invertebrates and in all classes of vertebrates. Cnidarians [coral (*12*)], crustaceans [water fleas and lobster (*13,14*)], mollusks [snails (*15*)], and echinoderms [starfish (*16*)] produce estradiol. The ubiquity of estradiol production in the animal kingdom suggests that estrogenically active chemicals may be evolutionarily conserved signals. It also suggests the possibility that all animals are sensitive to estrogens, whether endogenous or environmental.

Diethylstilbestrol (DES) is the archetype of a potent synthetic estrogen. The association of DES with adverse health effects in the offspring of DES-exposed women and laboratory animals has led to the hypothesis that environmental estrogens are contributing factors in adverse health trends in humans and animals (*17*). Although a direct association between human health trends and the presence of environmental estrogens has not been demonstrated and is still the subject of investigation (*18*), it has been suggested that decreased semen quality in men (*19*), decreased age of menarche (*20,21*), and an increased incidence of breast cancer in women (*22*) are associated

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Abbreviations used: 3-OH PCB, 2',4',6'-trichloro-4-biphenylol; 4-OH PCB, 2',3',4',5'-tetrachloro-4-biphenylol; DES, diethylstilbestrol; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; ER, estrogen receptor; MAPK, mitogen-activated protein kinase; P450, cytochrome P450; PAH, polyaromatic hydrocarbon; PCB, polychlorinated biphenyl; SHBG, sex hormone binding globulin; TBT, tributyltin; YES, yeast expressing estrogen receptor.

with exposure to environmental estrogens during development.

Several studies have shown that adverse reproductive effects observed in animals are linked to the presence of hormonally active chemicals in the environment. In the late 1970s and early 1980s, Fry and Toone (*23*) and Palmiter (*24*) separately showed that chlorinated insecticides, particularly DDT and its metabolites, had adverse impacts on the sexual development, reproductive capacity, and population size of birds. More recently, Sumpter and colleagues showed that male fish caught in several rivers in the United Kingdom had been feminized (*25*). Soon after the feminized fish were discovered, two common biodegradation products of alkyl polyethoxylate detergents, octylphenol and nonylphenol, were identified in river water. These compounds were shown to be estrogenic using *in vitro* assays indicating direct interaction with fish estrogen receptors (ERs) (*26*) and using *in vivo* assays demonstrating induction of vitellogenin (an egg yolk protein) in male fish (*25*). Alkylphenols in the rivers of the United Kingdom were suggested as a potential cause of fish feminization. Recently, however, the steroidal estrogens estradiol, estrone, and ethinyl estradiol (an oral contraceptive) have been quantified in U.K. rivers (*27*). This discovery has led to the hypothesis that the more potent steroidal estrogens also play a role in the feminization of fish found in U.K. rivers. Alkylphenols are 1000-fold less potent than estradiol in fish hepatocyte assays (*26*), but are 10,000-fold less potent than estradiol in yeast expressing recombinant human ER and 100,000-fold less potent in the mouse uterotrophic assay (*28,29*).

Jennings et al. (*30*) observed that the population of juvenile alligators in Lake Apopka, Florida, between 1981 and 1986 was severely reduced compared to earlier and subsequent years. Guillette et al. (*31,32*) later reported that this alligator population displayed a number of reproductive abnormalities similar to those seen in mice exposed to DES during development. These abnormalities included poorly organized testes and small phalli in juvenile males and large numbers of polyovular follicles and multinucleated oocytes in females. Guillette surmised that a chemical spill of dicofol and DDT and agricultural runoff had introduced hormonally active chemicals into the lake.

Bergeron et al. (*33*) have shown that the polychlorinated biphenyls (PCBs) 2′,4′,6′-trichloro-4-biphenylol (3-OH PCB) and 2′,3′,4′,5′-tetrachloro-4-biphenylol (4-OH PCB) cause sex reversal in red-eared slider turtles exposed during development. During normal development, temperature determines sex: eggs incubated at 26 or 31°C develop into males or females, respectively. Eggs incubated at a male-determining temperature can be sex-reversed by painting the eggs with estradiol. The PCBs also caused sex reversal.

Endocrine disruption in invertebrates has also been well documented (*16*). The most widely studied case of invertebrate endocrine disruption is imposex in gastropod mollusks. Female snails grow vas deferens and penises after exposure to very low levels (1 ng/liter) of tributyltin (TBT) (*15*). Several mechanisms of imposex induction have been proposed, including abnormal release of neuropeptides that stimulate penis development (*15*), inhibition of cytochrome P450 19 (aromatase), which converts testosterone to 17β-estradiol (*34*), and alteration of phase II (conjugating) enzyme (*35*) and reductase activities (*36*).

Normally, male gastropods resorb their penises during the nonreproductive season. When the appropriate temperature and day length occur, the pedal and cerebral ganglia begin secretion of neuropeptides that directly stimulate reproductive tissues to differentiate into a penis. By an unknown mechanism, TBT acts directly on these ganglia to induce secretion of neuropeptide, causing penis growth in males, juveniles, and females (*15*). Imposex can be so severe that the female's oviduct is completely blocked and can lead to her death (*34*). This worldwide phenomenon has caused local population extinctions in Europe (*37*). After international bans of TBT in the late 1980s and early 1990s, mollusk populations are beginning to recover (*38*).

Crustaceans have also been used as models of steroid endocrine disruption. Although many xenobiotics decrease fecundity or alter molting in crustaceans, the link to endocrine disruption as opposed to overt toxicity can only be confirmed in a few cases. Daphnids (water fleas) have reduced offspring production and altered testosterone metabolism after exposure to compounds such as DES, nonylphenol, pentachlorophenol, piperonyl butoxide, and TBT (*39–42*). Blue crabs exposed to TBT have elevated hydroxylation of testosterone (*43*). Additionally, blue crabs exposed to reproductive toxicants have decreased oocyte concentrations of lipovitellin, an indication that egg yolk proteins are not properly processed (*44*).

Environmental Signaling at the Cellular Level

Role of Bioavailability in Regulating Hormonal Activity

Availability of internal and external estrogenic signals to target tissues can be partially regulated by extracellular binding proteins in the plasma. Estradiol and DES differ in their interaction with α-fetoprotein, sex hormone binding globulin (SHBG) and albumin. The affinity of estradiol for mammalian SHBG and α-fetoprotein is in the nanomolar range, while the affinity of the synthetic estrogen DES is in the micromolar range (*45–49*). Because of their similar affinities for the ER, in the presence of ER and binding proteins, 20- to 100-fold more DES than estradiol will interact with ER. Not surprisingly, animals treated with DES show significantly greater increases in estrogen-specific responses than animals treated with an equivalent dose of estradiol (*50*). DES is also capable of acting as a transplacental carcinogen in humans and laboratory animals (*48*). Binding proteins in the maternal–fetal compartment normally protect the fetus from maternal steroid hormones. However, these proteins offer little or no fetal protection against DES (*48*).

These data suggest the hypothesis that steroidal and synthetic estrogens interact differently with extracellular binding proteins. In yeast expressing ER (YES), serum-binding proteins reduced the activity induced by phytoestrogens, steroidal estrogens, and synthetic estrogens (*51,52*). Purified human α-fetoprotein was more effective than SHBG or albumin at reducing reporter gene activity induced by estradiol or DES. Estradiol and phytoestrogen (genistein and coumestrol)-induced activity was suppressed to a far greater extent than the activity induced by DES or other synthetic estrogens, including *o,p′*-DDT and Kepone (*52*). The differential binding of phytoestrogens and synthetic estrogens with plasma proteins *in vivo* and in the YES assay is consistent with the hypothesis that animals and humans have been exposed to phytoestrogens throughout evolution, but synthetic estrogens represent a new challenge to the endocrine system.

Bioavailablity of synthetic estrogens may vary between species, depending upon the binding capacity of serum proteins. In the YES, human serum had a greater binding capacity for DES and *o,p′*-DDT than alligator serum (*51*). The degree to which serum proteins bind various estrogens may

determine the potential sensitivity or resistance of a species to the effects of certain exogenous estrogens. A fruitful area for future research will be to examine the binding of various estrogenic chemicals to sera from different species.

Bioavailability of chemical signals can also be regulated by cell membrane transport proteins controlling influx and efflux of chemicals. For instance, Bain and LeBlanc (*53*) have shown that the activity of the multidrug resistance transporter in human cells is affected by several pesticides. Other reports have shown that phytoestrogens modulate the activity of transport proteins including P-glycoprotein (*54*), and thereby mediate the cellular response to various drugs. Recently, Mahe et al. showed that special transport systems in yeast cells (ABC-cassette transporters) are involved in the active cellular import or export of estrogens (*55*). Clearly, the intracellular concentration of chemicals plays a major role in whether they exert activity.

Metabolism

Metabolism may also control the capacity of chemicals to mimic hormones. Steroid hormones are synthesized from cholesterol by a series of lyase and cytochrome P450 (P450) reactions. Specific P450 isozymes convert progesterone to testosterone, and aromatize testosterone to 17β-estradiol (*56*). In addition to activating steroids, P450s aid in elimination of all steroids by hydroxylating them and making them more hydrophilic. Other enzyme systems, such as reductases and transferases, are also involved in inactivation and elimination of steroids (*56*).

These enzyme systems metabolize estrogenic xenobiotics as well (*57*). Xenobiotics can upregulate P450 isozyme expression, leading to increased metabolism of these substrates and of endogenous steroids (*15*). P450s convert polyaromatic hydrocarbons (PAHs) and PCBs to more polar metabolites, which can aid in elimination. However, this modification may also drastically increase the estrogenicity of these compounds. Studies with *o,p′*-DDT demonstrated that rats pretreated with carbon tetrachloride, which supresses hepatic P450 activity, did not show the increase in uterine wet weight usually associated with DDT (*58*). It was subsequently shown that the hydroxylated metabolites created by P450 hydroxylation, 3-hydroxy, and 4-hydroxy *o,p′*-DDTs, are more potent than *o,p′*-DDT in increasing uterine weight in rats (*59*).

Hydroxylation increases the potency of certain PCBs as well. The hydroxylated PCBs, 3-OH PCB, and 4-OH PCB, function as estrogens *in vivo* and *in vitro* (*60,61*). Using 2 ,4, 6-trichlorobiphenyl and 2,3,4,5-tetrachlorobiphenyl (derivatives of the hydroxylated PCBs that do not possess a 4' hydroxyl group) and 2,4,4′,6-tetrachlorobiphenyl and 2,3,4,4′,5-pentachlorobiphenyl (derivatives of the hydroxylated PCBs that have a 4′ chlorine group), it was discovered that removal of the 4′-hydroxyl group or replacement of the 4′-hydroxyl group with a chlorine completely eliminated estrogenic activity and ER binding activity *in vitro* (*61*).

Several phytoestrogens also require appropriately positioned hydroxyl groups for ER binding and activation (*62–65*). For example, genistein has substantially greater ER binding capacity and estrogenic activity than daidzein. Interestingly, these two chemicals differ only by the presence of the 4′ hydroxyl group in genistein but not daidzen. Although the presence of hydroxyl groups on various chemicals appears to correlate with their ability to interact with ER, it is still not understood how the ER preferentially responds to hydroxylated chemicals.

Estrogenic compounds compete as substrates for reductases and phase II enzyme systems as well. The glucosylation or methylation of key hydroxyl groups on phytoestrogens decreases ER binding activity and estrogenic activity. The balance of the metabolic processes of hydroxylation, glucosylation, and methylation may determine the extent to which phytoestrogens and other synthetic chemicals are hormonally active.

Interaction of Chemicals with Hormone Receptors

Many environmental estrogens appear to exert their effects by interacting with the ER. Phytoestrogens (*62–65*), hydroxylated PCBs (*60,61*), and alkyl phenols (*26*) are bound by the ER and can activate receptor-mediated processes. However, the estrogenic responses produced and the ER affinity of these chemicals are 100- to 10,000-fold lower than estradiol. Limited work has examined whether synthetic chemicals that are bound by ER can function as antiestrogens in the presence of estradiol or other estrogens. Some chemicals such as the dioxins appear to exert antiestrogenic activity by interacting with the aryl hydrocarbon receptor and indirectly decreasing the activity of ER (*66*). Some phytoestrogens appear to function as anti-estrogens but their mechanism of action is still unclear (*62*).

The recent cloning of another isoform of ER (ERβ) indicates a greater complexity of estrogen action than previously appreciated (*67*). The recent work by Paech et al. (*68*) indicates that ERβ signaling depends upon the specific ligand and the DNA response element bound. Using reporter gene assays in human cells, these investigators showed that estradiol-bound ERβ inhibits transcription at a nonclassical AP1 response element, whereas antiestrogen (raloxifene, tamoxifen, or ICI164,384)-bound ERβ stimulates transcription. Interestingly, rat ERβ binds the phytoestrogens genistein and coumestrol with a 10-fold higher affinity than human ERα, suggesting that different isoforms of ER may mediate different responses to environmental estrogens (*69*).

Other steroid hormone or nuclear receptors have been shown to interact with environmental chemicals. Kelce et al. (*70*) demonstrated that *p,p′*-DDE, a metabolite of the insecticide DDT, was bound by the androgen receptor and inhibited its activity *in vitro* and *in vivo*, i.e., *p,p′*-DDE functioned as an antiandrogen. Other antiandrogens include some PAHs (*71*), linuron (*72*) and vinclozolin (*73*). We have recently shown that several chemicals including octyl- and nonylphenol, pentachlorophenol, and lindane function *in vitro* as antiprogestins by interacting with the progesterone receptor and inhibiting the activity of progesterone or the synthetic progestin R5020 (*74,75*). Some metabolites of DDT also function as antiprogestins (*76*), suggesting that this class of chemicals is capable of interacting with a broad spectrum of hormone receptors. The insecticide methoprene has recently been shown to function *in vitro* as a retinoid mimic with a homodimer of the retinoid X receptor (*77*). Current evidence suggests that other nuclear receptors are sensitive to environmental chemicals, including the glucocorticoid and thyroid receptors. Interaction of environmental chemicals with glucocorticoid receptors may be particularly relevant as several reports have indicated a correlation between the presence of environmental chemicals and decreased immune activity (*78*). Environmental signaling via the thyroid receptor may be relevant to reported deficiencies in thyroid-mediated nervous system development in rats and humans (*79–84*).

Interaction with Cell-Signaling Pathways

Environmental hormone mimics also influence receptor independent cell-signaling pathways. For example, high concentrations

of *p,p'*-DDD increased the free intracellular calcium concentration in rat myometrial smooth muscle cells (*85*). These results are not surprising as DDT, of which *p,p'*-DDD is a metabolite, affects the sodium channel in insects. The modern-use pesticide endosulfan blocks gamma-amino butyric acid (GABA)-gated chloride ion channels. Some PCBs affect calcium homoeostasis and protein kinase C activation (*86*). Recent reports have indicated that some chemicals such as peroxisome proliferators and chlorinated insecticides activate the kinase activity of mitogen-activated protein kinase (MAPK) (*87,88*). Finally, the observation that β-hexachlorocyclohexane induces some ER-specific responses but does not interact with ER (89) suggests that this chemical may activate some signaling pathways that increase the activity of ER in a ligand-independent manner. This hypothesis is based on previous data demonstrating that ER-specific responses can be produced in an estrogen-independent manner by treating cells with growth factors (*90*). The mechanism for this effect may involve the activation of MAPK and possibly other cell-signaling proteins. Thus, some chemicals may regulate hormonal responses by directly modulating cell-signaling pathways rather than interacting with hormone receptors.

Implications of Environmental Signaling

In this short review, we have developed the concept of environmental signaling, which we think provides a biological context in which to view endocrine-disrupting chemicals in the environment. We have focused on signaling systems at the organismal and cellular levels. In doing so we did not focus on possible human health effects of endocrine disruption. Based on the concept of environmental signaling, synthetic chemical hormone mimics could plausibly be associated with recent adverse health trends in humans as well as animals. Sensitivity to endogenous and exogenous chemical signals has evolved as a means by which organisms respond to physical or biological stimuli in the environment. This sensitivity makes organisms vulnerable to inadvertent signals from anthropogenic chemicals.

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